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Antibacterial Activity of Cocoa Leaf Extract *Theobroma cacao* L. Against Acne-Causing Bacteria *Cutibacterium acnes* and *Staphylococcus epidermidis*

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Abstract

Background: Cocoa (*Theobroma cacao* L.) plantation waste and cocoa leaves are increasingly abundant, with efforts to increase cocoa production in the form of cuttings. Ladongi District in Kolaka Regency, Southeast Sulawesi, as the region that produces the most cocoa beans, is also not spared from this. The utilization of cocoa leaves is not optimal, even though their phytochemical components have the potential as antibacterial. This study aimed to determine the antibacterial activity of cocoa leaf extract (*Theobroma cacao* L.) against acne-causing bacteria, namely *Cutibacterium acnes* and *Staphylococcus epidermidis*. **Method:** The disc diffusion method is used (Kirby Bauer). The research data were analyzed using the Shapiro-Wilk normality test, the ANOVA test with the SPSS 25.0 program, and Duncan's follow-up test to determine a significance value of 5%. **Results:** Cocoa leaf extract has the potential as an antibacterial against acne-causing bacteria, *Cutibacterium acnes,* and *Staphylococcus epidermidis*, with the best inhibitory concentrations of 25%, 1.90 ± 0.58 cm and 2.20 ± 0.58 cm, respectively. **Conclusion:** Cacao leaf extract (*Theobroma cacao* L.) with ethanol solvent has the potential as an antibacterial against acne-causing bacteria.

Keywords: Acne; Antibacterial; Cocoa leaves, Extract

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Introduction

Cocoa (*Theobroma cacao* L.) is one of the plantation commodities whose role is quite crucial for the Indonesian economy. One of the largest cocoa-producing regions in Indonesia is Southeast Sulawesi, with a productivity of 137,737 kg/ha with an area of 259,739 ha in 2019 Ladongi District, located in Kolaka Regency, Southeast Sulawesi, is the area that produces the most cocoa beans. One of the efforts to increase cocoa production is implementing Good Agriculture Practices (GAP) by trimming the shape (Ermiati *et al.*, 2014). Cocoa leaves obtained from pruning have yet to be utilized optimally, only used as animal feed (Gunawan *et al.*, 2017). In addition, cocoa processing focuses only on the fruit and the seeds' skin. Based on phytochemical components (Irma & Reza, 2021), cocoa leaves can be processed into products with other benefits and high economic value (Supriyanto, 2014), such as an antibacterial agent (Ningsih *et al.*, 2016).

Indonesia, as a tropical country, causes its people to often suffer from acne. At the same time, the needs and lifestyles of today's society are very concerned about skin health and physical appearance. *Cutibacterium acnes* and *Staphylococcus epidermidis* are the most common bacteria in acne vulgaris (Sari *et al.*, 2021), and *Staphylococcus* sp. could trigger acne (Imasari & Ficka, 2020). Treatment of acne using antibiotics in the long term causes

bacterial resistance. It is necessary to carry out a chemoprevention strategy and the development of therapy, one of which is utilizing natural extracts. This study aims to determine the potential of cacao leaves (*Theobroma cacao* L.) as a source of antibacterial acne caused by *Cutibacterium acnes* and *Staphylococcus epidermidis*. The benefit of this study is to provide information on the antibacterial activity of cocoa leaf extract against acne caused by *Cutibacterium acnes* and *Staphylococcus epidermidis* bacteria. In addition, this research is a form of implementing the Green Economy in Indonesia, especially Kolaka, Southeast Sulawesi, so that it can be used as a reference in the manufacture of antibacterial cosmetic products that cause acne, *Cutibacterium acnes*, and *Staphylococcus epidermidis*.

Methods

Materials

The research was conducted at the Basic Laboratory of Universitas Sembilanbelas November Kolaka and the Microbiology Laboratory of the Politeknik Bina Husada Kendari. The tools used were sample paper, coffee roll paper, aluminum foil, cotton, 10 ml vials, 80 mesh test sieve, paper scissors, rotary evaporator, analytical balance, vessel, water bath, petri dish, and micropipette. The materials needed include PA Merck ethanol, test bacteria, Nutrient Broth media (Merck), Nutrient Agar media (Merck), and Mueller Hinton Agar (MHA) media. A 10% DMSO, 0.9% NaCl, methylated spirits, 70% alcohol, and Mc. Farland.

Procedure

The first stage is sample preparation. Cocoa leaves were obtained from cocoa plantations in Ladongi District, Kolaka, Southeast Sulawesi.

Step 1: Sample Preparation. Cocoa leaves were sorted wet and washed using running water and then dried, then sorted dry. The dried Simplicia was then crushed and sieved using a Sieve Mesh No. 80. Cocoa leaves were extracted using the maceration technique. Five hundred grams of cocoa leaf Simplicia powder was put into a maceration vessel, then 75% ethanol solvent was added with a ratio of 1:3.

Step 2: Extraction using the maceration method. Maceration was carried out for three days. The obtained macerate was then evaporated using a rotary evaporator to separate the cocoa leaf extract and the solvent to get a thick section.

Step 3: Antibacterial Assay. The antibacterial activity of the thick cocoa leaf extract was tested using the disc diffusion method (Kirby Bauer). First, each culture loop of *Cutibacterium acnes* and *Staphylococcus epidermidis* was cultured in NB (Nutrient Broth) and NA (Nutrient Agar). One bacterial rod from the NA medium was suspended in a tube containing 10 ml of 0.9% NaCl. This step was carried out until the turbidity was equal to the standard turbidity of 10 ml of 0.5 Mc Farland solution (liquid culture, which has a population of 1x107 CFU/ml to 1x108 CFU/ml). 20µL of suspended bacteria was taken from the tube, then spread on the surface of the MHA media with a spreader. The antibacterial activity test of cocoa leaf extract used various extract concentrations of 25%, 50%, 75%, and 100%.

The stock extract was diluted with 10% DMSO solvent to obtain each of these concentrations. Each concentration of the section was made in 1 mL volume. 25% concentration requires 0.25g extract, 50% concentration requires 0.5g extract, 75% concentration requires 0.75g extract, and 100% concentration requires 1g extract. Especially for the attention of 100% does not need to be diluted with DMSO 10%. Each extract concentration was added to 20µL disc paper with a diameter of 5 mm. Sterile disc paper that has been dripped with extract and then placed on the surface of the media with clean tweezers by pressing down to ensure contact between the disc paper and the surface of the media. Each treatment was repeated three times. Dripping paper discs containing 20µL of Chloramphenicol and Clindamycin solutions prepared positive controls. The negative control was designed by dripping the disc paper with the extracting solvent (DMSO%). Furthermore, the petri dish was incubated at 37°C for 24 hours. The diameter

of the zone of inhibition against *Cutibacterium acnes* and *Staphylococcus epidermidis* was measured using a caliper with the following formula:

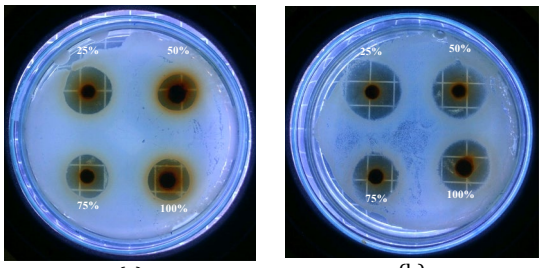
$$\text{Zone of Inhibition} = \frac{(\mathbf{DV-DC})+(\mathbf{DH-DC})}{\mathbf{2}}$$

Description:
DV: Diameter of Vertical
DC: Diameter of Disc (5mm)
DH: Diameter of Horizontal

The results were analyzed using the normality test Shapiro-Wilk and ANOVA test using SPSS 25.0 Program and Duncan's follow-up test to determine the 5% significance value.

Result

The maceration results were obtained in 1200 mL of filtrate based on the extraction process. The filtrate is then evaporated. The result of filtrate evaporation was obtained from a thick extract with a brown color of 15.06 g. Then the section was stored in a vial and a refrigerator at 4^0C. The results of the antibacterial activity test showed that cocoa leaf (*Theobroma cacao* L.) extract affected the growth of acne-causing bacteria *Cutibacterium acnes* and *Staphylococcus epidermidis.* The presence of an inhibition zone around the paper disc evidence this effect.



(a) (b)

Figure 1. Antibacterial activity of *Theobroma cacao* L. cocoa leaf extract with varying concentrations against *Cutibacterium acnes* (a) and *Staphylococcus epidermidis* (b)

The diameter of the inhibition zone of *Theobroma cacao* L. cocoa leaf extract with varying concentrations against *Cutibacterium acnes* and *Staphylococcus epidermidis* is shown in Table 1.

Table 1. Inhibitory Zone Diameter of Cocoa Leaf Extract Against Test Bacteria

Cutibacterium acnes	
Treatment	**Average of Inhibitory Zone Diameter (cm) + SD**
Negative control (DMSO10%)	0,00±0,00[a]
25% concentration	1,90±0,58[b]
50% concentration	1,63±0,58[c]
75% concentration	1,23±0,58[d]
100% concentration	1,03±0,58[d]
Positive control (Clindamycin)	3,53±0,58[e]
Staphylococcus epidermidis	
Treatment	**Average of Inhibitory Zone Diameter (cm) + SD**
Negative control (DMSO10%)	0,00±0,00[a]
25% concentration	2,20±0,58[b]
50% concentration	1,80±0,58[c]
75% concentration	1,50±0,58[d]
100% concentration	1,60±0,58[d]
Positive control (Chloramphenicol)	3,55±0,58[e]

Note: Notation a, b, c, d, and e shows that the results obtained are significantly different based on Duncan's test with a significance level of 0.05

Based on Table 1. it can be seen that each concentration of the extract affects bacterial growth because an inhibition zone is formed. These results indicate a significant difference in the effect at each concentration. Ethanol extract from cocoa leaves (*Theobroma cacao* L.) affected the growth of *Cutibacterium acnes* and *Staphylococcus epidermidis* at all concentrations, 25%, 50%, 75%, and 100%. However, the most significant inhibition of bacterial growth was shown at an extract concentration of 25% for the two test bacteria, *Cutibacterium acnes,* and *Staphylococcus epidermidis,* with the diameter of the inhibition zones 19mm and 22mm, respectively. Antibacterial activity of cocoa leaf extract against *Cutibacterium acnes* at 100% concentration has the lowest diameter of inhibition zone 1,03±0,58 cm, and against *Staphylococcus epidermidis* at 75% concentration has the most inferior diameter of inhibition zone 1,50±0,58 cm. At concentrations of 25%, 50%, and 75%, the results were significantly different. Antibacterial activity of cocoa leaf extracts against *Staphylococcus epidermidis* at 75% and 100% concentrations. There was no significant difference in effect. The negative control in this study was DMSO. No inhibition zone was formed in the adverse control treatment. The positive control in this study for *Cutibacterium acnes* was Clindamycin and Chloramphenicol for *Staphylococcus aureus*. The inhibitory zone results for Clindamycin and Chloramphenicol positive control were 3.53±0,58 and 3,55±0,58 cm.

Discussion

The extraction uses the maceration method. The reason for using the maceration method is that it does not require heating to prevent the organic matter from destruction. In addition, the extract obtained by the maceration method was more optimal than other methods (Mandhaki *et al.,* 2021).

The negative control in this study was DMSO. No inhibition zone was formed in the adverse control treatment, 0,00±0,00 cm. It is proven that using DMSO 10% does not affect bacterial growth. DMSO is a universal solvent used to dissolve various compounds (Suryani *et al.*, 2019). In general, DMSO can dissolve the extract completely. Allows the section to diffuse into the media well so that the process of inhibiting bacterial growth can be optimal. The positive control in this study for *Cutibacterium acnes* was Clindamycin and Chloramphenicol for *Staphylococcus aureus*. Clindamycin is used based on research conducted by Mandhaki *et al.,* 2021. The mechanism of action of Clindamycin in inhibiting bacterial growth is by causing bacteria to make errors in reading the genetic code, blocking site A on the ribosome, and having the ability to cut the elongation step in the peptide chain. It can block the attachment of oligosaccharide chains to the ribosome glycoproteins (Mazidah *et al*., 2014). On the other side, the mechanism of action of chloramphenicol is to inhibit bacterial protein synthesis by binding in reverse to the 50S subunit of the ribosome to inhibit the formation of peptide bonds. Kloramfenikol merupakan antibiotik spektrum luas dengan efek bakteriostatik terhadap bakteri gram positif dan gram negative (Tjay & Rahardja, 2015).

The size of the inhibition zone indicates the effectiveness as an antibacterial of the cocoa leaf extract. The inhibition zone diameter category (Surjowardojo *et al*., 2015) is 5 mm weak, 6–10 mm moderate, 11–20 mm strong, and 21 mm very strong. However, when compared with the antibacterial activity of cocoa pod peel extract (Adha & Ibrahim, 2021) against *Propionibacterium acnes*, cocoa leaf extract had better inhibitory activity. Furthermore, previous research by Mandhaki *et al*., 2021 showed that the cocoa leaf fraction obtained from the ethanol extract of cocoa leaves also showed antibacterial activity against *Staphylococcus aureus*, a member of the *Staphylococcus* genus.

The higher the concentration of the extract, the higher the concentration of bioactive substances and the higher the antibacterial effect. However, concentrations from 50% and 75% to 100% did not show an increase in the inhibition zone. The inhibition zones formed at concentrations of 50%, 75%, and 100% were smaller than the concentrations of 25%. This can be caused by the attention of the extract with concentrations of 50%, 75%, and

100%, which is more concentrated, thereby reducing the diffusion power on Mueller Hinton Agar (MHA) media. Thus, the formation of the inhibition zone is getting smaller even though the concentration of the extract increases because fewer bioactive substances can diffuse into the medium.

The results cocoa leaves (*Theobroma cacao* L.) test contain secondary metabolites (flavonoids, alkaloids, tannins, saponins, steroids, and glycosides) (Irma & Reza, 2021). The antibacterial mechanism of flavonoid compounds is to damage the cytoplasmic membrane because these compounds can cause leakage of essential metabolites and activate the enzymatic system of bacteria. When the membrane is broken, nucleotides and amino acids are out, preventing the drug enters the cell and may kill bacteria (Prajitno, 2007). The mechanism of alkaloid compounds as antibacterial is to avoid the components which form peptidoglycan in bacterial cells so that the cell wall layers are not fully developed and cause cell death. In addition, the components of alkaloids are known as DNA Interchelators and inhibit the topoisomerase enzyme from bacterial cells (Ningsih *et al.,* 2016). Saponins can be used as antibacterial agents by lowering the surface tension of bacterial cell walls (Hassan, 2008). The antimicrobial mechanism of Tannins is lysing tannins bacterial cell walls, inhibiting the formation of the bacterial cell walls, and killing bacterial cells (Ngajow, 2013)

The research conducted by Adha & Ibrahim (2021) showed that cocoa leaf bark extract showed antibacterial activity against Propionibacterium acnes, currently called *Cutibaterium acnes*. Compounds owned in part of a plant can also be in other features, such as leaves or fruit skin.

Conclusions

Cocoa leaf extract (*Theobroma cacao* L.) with ethanol solvent has the potential as an antibacterial against acne-causing bacteria, *Cutibacterium acnes,* and *Staphylococcus epidermidis,* with respectively, the best inhibitory concentration of 25%, 1,90±0,58 cm, and 2,20±0,58 cm.

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Declaration statement

The authors reported no potential conflict of interest

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